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Palomo, Alejandro; Smets, Barth F.

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DTU Environment
Department of Environmental Engineering

Technical University of Denmark

Genomic and ecological variation in comammox Nitrospira populations

Alejandro Palomo* **and Barth F. Smets**
Department of Environmental Engineering, Technical University of Denmark, Kgs Lyngby, Denmark.

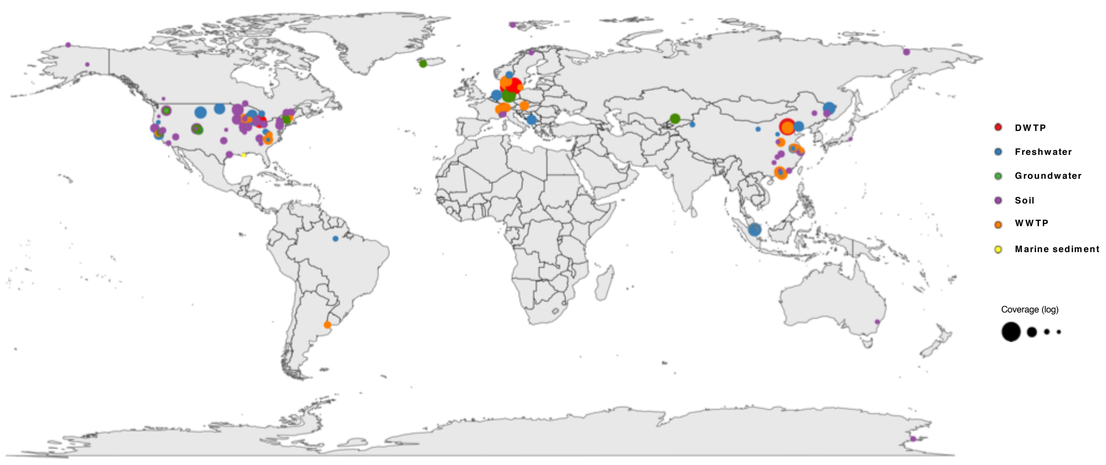
Introduction and Objectives

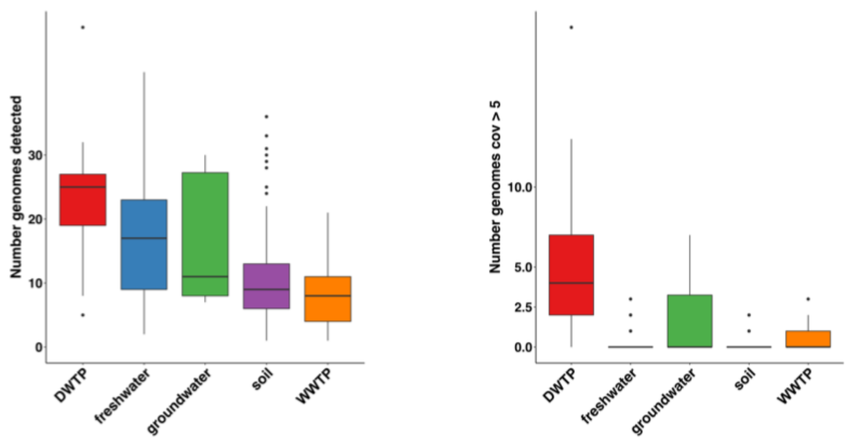
- ✓ Complete ammonia oxidizing (comammox) bacteria belong to *Nitrospira*, an extremely diverse genus of nitrite oxidizing bacteria.
- ✓ Described comammox *Nitrospira* genomes belong to *Nitrospira* lineage II, and comprise two clades (clade A and B) based on the phylogeny of their ammonia monooxygenases.
- ✓ *Nitrospira* spp. are widespread in both natural and engineered ecosystems where nitrogen cycling occurs; multiple species frequently coexist.
- ✓ However, the forces driving differentiation and diversification in *Nitrospira* populations remain poorly understood.
- ✓ The purpose of this study was to investigate the distribution of comammox and other *Nitrospira* spp. across different environments and geographical locations.

Approach

- ✓ 55 different metagenome-assembled genomes (MAG) of *Nitrospira* were retrieved from 13 Danish groundwater-fed rapid sand filters and multiple publicly available metagenomes by combining different binning tools (MetaBAT[1], MaxBin2[2] and CONCOCT[3]) followed by binning refinement (Binning refiner[4], DAS Tool[5] and metaWRAP[6]) and dereplication (dRep[7]).
- ✓ These *Nitrospira* MAGs together with 37 published *Nitrospira* genomes were clustered (genomes with ANI ≥ 95% were classified as member of same species) to create a database of *Nitrospira* species.
- ✓ The abundance of each *Nitrospira* species across 1000 metagenomes was assessed using MIDAS[8].
- ✓ Distribution patterns of *Nitrospira* genomes across the metagenomes was visualized through principal component analysis (PCA) .

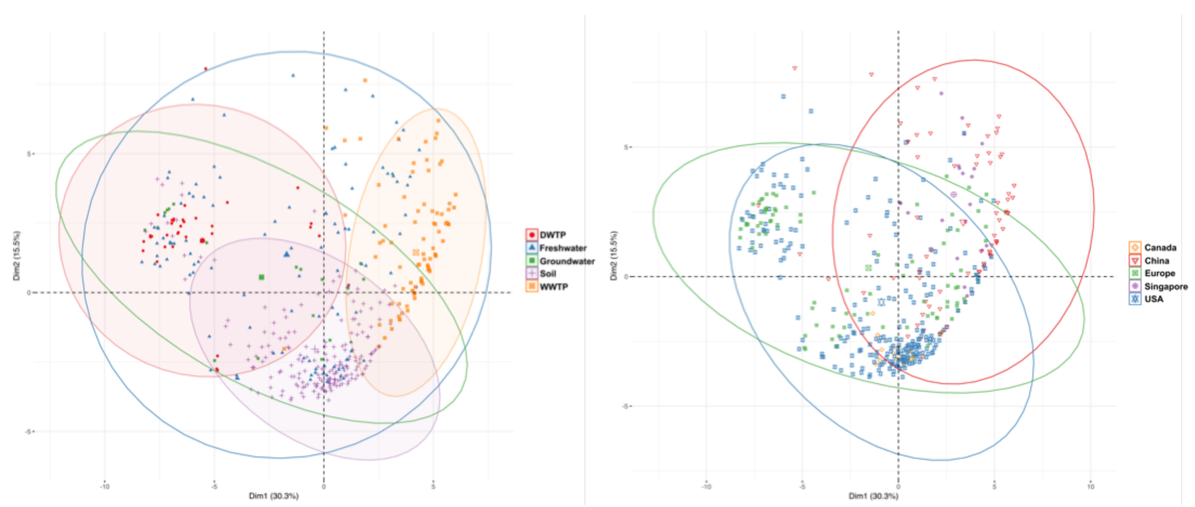
Global distribution of *Nitrospira* spp.

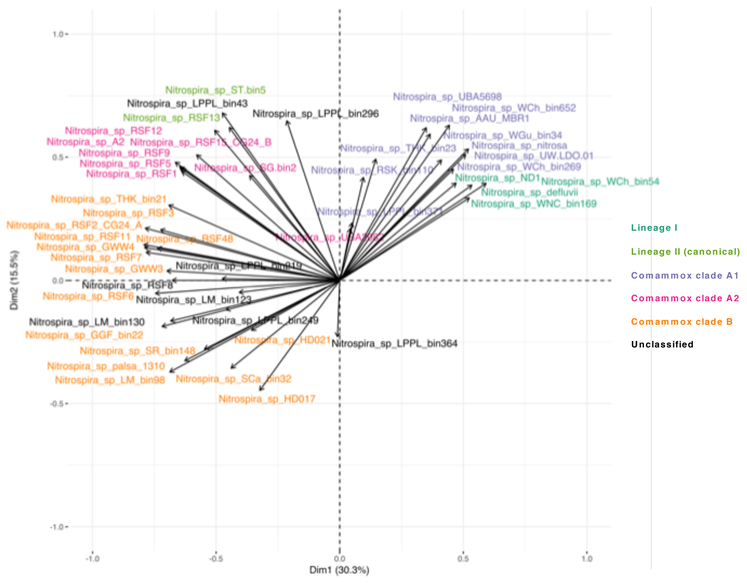




More *Nitrospira* genomes were detected in metagenomes from DWTP (drinking water treatment plant) and freshwater samples compared to soil and WWTP (wastewater treatment plant) samples. Only DWTP metagenomes contained multiple abundant *Nitrospira* genomes (coverage > 5).

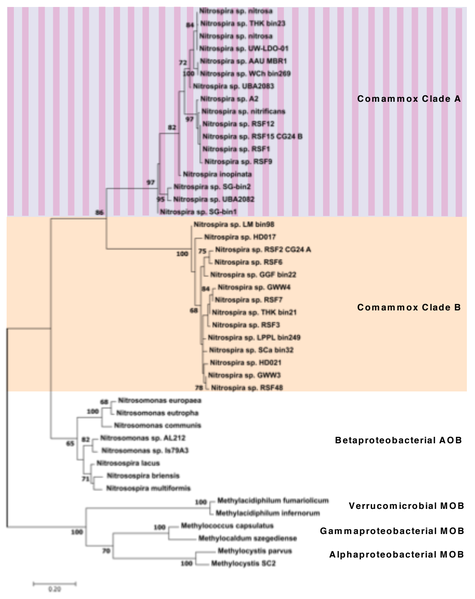
Genomic and diversity patterns of *Nitrospira* spp.



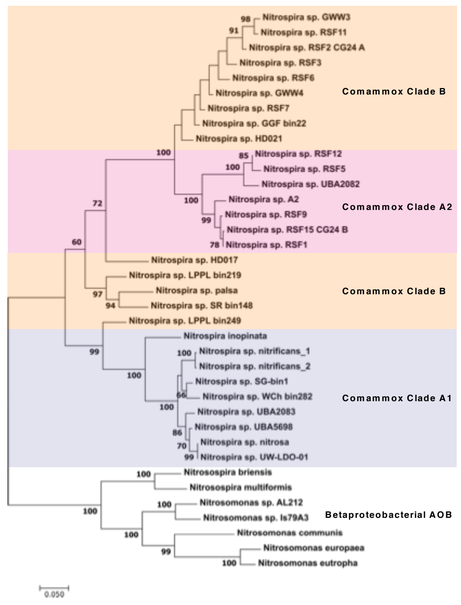


Comammox *Nitrospira* Clade A genomes (purple and pink) form two different clusters: CladeA1 genomes (purple) co-occur with non-comammox *Nitrospira* lineage I genomes and are present in WWTP samples; CladeA2 genomes (pink) are present in DWTP, groundwater and freshwater samples.

AmoA-based phylogeny



HaoA-based phylogeny



AmoA and HaoA sequences phylogenies are not congruent. While AmoA phylogeny separates comammox *Nitrospira* into two clades (A and B), HaoA phylogeny suggests existence of a subdivision within clade A (CladeA1 and CladeA2)

Conclusions

- ✓ *Nitrospira* genomes were detected in metagenomes from very different environments and geographical locations; a higher abundance was observed in DWTP metagenomes.
- ✓ Environment more than geography separates different *Nitrospira spp.:* WWTP metagenomes separate strongly from DWTP metagenomes based on presence and type of *Nitrospira* genomes.
- ✓ *Nitrospira* spp. affiliated to the same lineage or clade inhabit similar environments.
- ✓ Environmental partitioning within the comammox Clade A was detected. A similar separation was observed based on the phylogeny of hydroxylamine oxidoreductase subunit A amino acid sequences.

alpal@env.dtu.dk
@alejandropg11
@Metlab_DTU

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